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Engraftment Syndrome in Breast Cancer Patients After Stem Cell Transplantation Is Associated With Poor Long-term Survival

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ABSTRACT

An autoaggression graft-versus-host (GVHD)-like syndrome or engraftment syndrome (ES) presenting with skin rash, fever, and other clinical findings can accompany the early phase of engraftment after autologous peripheral blood stem cell (PBSC)/bone marrow (BM) transplantation. Because ES was suggested to be analogous to GVHD, we have investigated whether ES was associated with any graft-versus-tumor effect that would affect disease progression and survival in breast cancer patients. Eighty-five consecutive patients who received BM/PBSC transplantation for breast cancer (stages II-IV) between July 1991 and July 1997 with minimum 2-year follow-up were studied. Median follow-up time was 892 days (range, 106-2913 days). Thirty-three patients (39%) developed ES. The incidence of relapse/progressive disease for the whole cohort was 61% and was similar in patients who developed ES compared with those who did not. However, there was an increased rate of mortality observed among the patients who had developed ES versus those who had not, although it was statistically not significant, (52% versus 31%, respectively; log rank, $P = .08$). Increased mortality rates due to disease progression were seen in all patients with ES regardless of their disease stage. In relapsed patients, median survival time after transplantation was 586 days for those with ES versus 847 days for those without ES, and the mortality rate was 85% (17/20) versus 51% (16/31) ($P = .008$) for those with or without ES, respectively. Visceral (lung, liver, brain, adrenal) or multiple-site relapses were observed in 85% of patients with ES versus 52% without ES ($P = .01$). In conclusion, whereas there was no effect of ES on relapse rate, a surprisingly significant increase in disease-related mortality rates among relapsed breast cancer patients with ES was found. Thus, patients with ES should be considered for close follow-up and further therapy posttransplantation.

KEY WORDS

Engraftment syndrome • Autoaggression syndrome • Breast cancer • Autologous stem cell transplantation • Graft-versus-host disease

INTRODUCTION

High-dose chemotherapy followed by autologous bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) has been used for treatment of various hematological and nonhematological malignancies. Relapse after autologous transplantation remains the major cause of morbidity and mortality after these treatments [1]. Graft-versus-host disease (GVHD) is a complication of allogeneic BMT and is thought to be the response of donor lymphocytes against foreign histocompatibility antigens of the recipient [2]. A syndrome that clinically resembles GVHD has been reported in up to 10% of patients following autologous BMT [3-5]. The graft-versus-leukemia (GVL) and graft-versus-tumor (GVT) effects are well-described immunologic responses that contribute substantially to the curative capacity of allogeneic BMT above that produced by the high-dose preparative regimens in leukemia and lymphoma patients [6-8]. Relapse rates were shown to be higher in patients with hematological malignancies who receive T-cell–depleted allogeneic [9], autologous, or syngeneic marrows compared with those receiving unmanipulated allogeneic transplants [10,11]. Studies aiming at inducing GVHD after autologous stem cell transplantation have been conducted to evaluate GVHD's potential antitumor activity

Table 1. *Patient Characteristics*

No. of patients	**84**
Age, median (range), y	**46 (20-64)**
Follow-up, median (range), d	**870 (106-2913)**
Disease stage, n	
II	**11**
III	**28**
IV	**45**
Stem cell source, n	
Peripheral blood stem cells	**67**
Bone marrow/peripheral blood stem cells	**17**
No. of mononuclear cells infused, median (range) $\times 10^8$/kg	**6.10 (0.88-14.1)**

against various malignancies, including lymphoma, multiple myeloma, acute myeloid leukemia, and breast cancer. Cyclosporin, [12-14], interferon γ [15], and interferon α [16,17] have all been used to facilitate the development of autologous GVHD with limited or no success.

Engraftment syndrome (ES), also known as autoaggression syndrome, is a well-described syndrome consisting of development of skin rash, noninfectious fever, and other clinical findings including platelet refractoriness and pulmonary symptoms (hypoxia, pulmonary alveolar hemorrhage) at the time of hematological recovery and engraftment after autologous BMT [18-21]. These manifestations, as well as histological features of GVHD on skin biopsies, have led us and others to suggest that ES is analogous to autologous GVHD [18,19]. ES has been noted to occur with greater frequency in breast cancer patients [18]. Therefore, in this study, we investigated whether development of ES is associated with any GVT effect, which would be reflected in lower relapse and mortality rates after autologous stem cell transplantation in breast cancer patients.

PATIENTS AND METHODS

Study Population

Retrospective data were collected by chart review of 85 consecutive patients with breast cancer who underwent high-dose chemotherapy followed by autologous stem cell transplantation. All patients were treated in our institution between July 1991 and July 1997. Of these 85 patients, 1 patient died on day 41 due to pulmonary toxicity (pulmonary alveolar hemorrhage) and was excluded from the analysis because the study included only patients with 2-year minimum follow-up. Thus, patients undergoing transplantation within the last 2 years were also excluded. Table 1 shows patient characteristics. Median follow-up of the patients was 870 days (range, 106-2913 days). Median age was 46 years (range, 20-64 years). All patients had staging work-ups prior to transplantation, including computed tomographic (CT) scans of the chest, abdomen, and pelvis; CT scan or magnetic resonance imaging of the brain; bone scans; and BM biopsies. Disease stages at the time of transplantation were as follows: 11 patients in stage II, 28 in stage III, and 45 in stage IV. Other data were collected regarding prior therapy (characterized by number of prior treatments), stem cell dose, time to relapse, pattern of relapse, mortality, and overall survival. All patients were treated with the same conditioning regimen (STAMP V), which included cyclophosphamide, 6000 mg/m^2; carboplatin, 800 mg/m^2; and thiotepa 500 mg/m^2; all given by continuous infusion over 96 hours. All patients who suffered posttransplantation relapse received standard therapy.

Stem Cell Harvest and Transplantation

PBSC mobilization was performed using granulocyte colony–stimulating factor (G-CSF) alone (10 μg/kg) or chemotherapy and G-CSF (5 μg/kg). Cyclophosphamide, 2 gm/m^2, was used for chemotherapy mobilization. G-CSF was administered subcutaneously starting 48 hours after the administration of priming chemotherapy and continued until completion of apheresis. Patients underwent 16- to 20-L leukapheresis using the Cobe Spectra blood cell separator (Cobe Laboratories, Lakewood, CO). Median cell dose infused was 6.1×10^8 mononuclear cells (MNCs)/kg (range, $0.88\text{-}14.1 \times 10^8$ MNCs/kg). The median dose of CD34$^+$ cells infused (data available for 34 patients only) was 3.33×10^6/kg body wt (range, $1.01\text{-}7.27 \times 10^6$/kg body wt). Seventeen patients received BM or both BM and PBSCs, and the remainder (67 patients) received only PBSCs.

Engraftment Syndrome

The autoaggression syndrome or ES was defined as previously reported [18]. Typically, the syndrome appears approximately at the time of engraftment after autologous stem cell transplantation, usually includes maculopapular rash and noninfectious fever (up to 40°C), and may also include thrombocytopenia with platelet transfusion refractoriness, diarrhea, and diffuse pulmonary infiltrates with shortness of breath and hypoxemia [18-21]. Skin biopsies may show changes typical of acute GVHD [18]. The severity of the syndrome varies, and sometimes it resolves spontaneously. Patients with persistent fever and worsening rash or any other manifestations responded to treatment with methylprednisolone, 1 mg/kg per day, followed by a quick taper over 10 to 14 days with resolution of symptoms. In most patients, more than 50% of their body surface was covered with an erythematous maculopapular rash. Patients who developed pulmonary symptoms and hypoxia underwent bronchoscopy to rule out infectious etiology and were treated with high-dose methylprednisolone as previously described [18]. All occurrences of fever were investigated to rule out infection, using routine blood and urine cultures, chest x-rays, and any other studies indicated after physical examination. G-CSF was discontinued upon appearance of ES and any evidence of granulocyte recovery.

Outcome and Statistical Analysis

Patients who developed symptoms suggestive of ES were identified and compared with those who did not have ES. The relapse and survival rates were compared between the 2 groups (with or without ES) using the Fisher exact test. Overall survival rate was compared by time-to-event analysis using the Kaplan-Meier method. Statistical software (Statsoft, Groningen, the Netherlands) was used for analysis. The site of relapse (visceral versus nonvisceral) was also compared for both groups using the Fisher exact test. In order to investigate whether ES is an independent risk

Table 2. *Overall Relapse Rate for Breast Cancer Patients After Autologous Transplantation*

Disease Stage	All Patients	Patients With Relapse/Progressive Disease, n (%)
II	11	2 (18)
III	28	11 (39)
IV	45	38 (84)
Total	84	51 (61)

factor for bad outcome, univariate as well as multivariate analyses were conducted using Cox Regression model SAS software version 6 (SAS Institute Inc, Cary, NC). Variables were included on an a priori basis, such as estrogen receptor (ER) status, the presence of visceral metastases at the time of transplantation, prior therapy, and others. We performed both a forward-stepping as well as best-possible-subsets analysis for the regression modeling.

RESULTS

Patients With ES

Thirty-three patients (39%) developed ES. The manifestations were maculopapular rash (n = 29) involving the body and extremities and temperature elevation (up to 40°C) within 1 to 2 days after an increase in leukocyte count from a nadir of 100 to 200/μL (n = 31) without evidence of infection. Twenty-three of these patients with a persistent fever of >38.5°C and worsening itchy rash required treatment with methylprednisolone at 1 mg/kg per day for 3 days, followed by quick taper over 2 weeks with resolution of symptoms. Three patients had pulmonary alveolar hemorrhages in addition to skin rash and high fever, and responded to high-dose methylprednisolone. None of the patients died from ES, and the response to steroids was usually dramatic within 24 hours. There was no effect of $CD34^+$ stem cell dose on development of ES. Data available for 34 patients showed median $CD34^+$ stem cell doses of 3.0×10^6/kg versus 3.62×10^6/kg in patients with and without ES, respectively.

Effect of ES on Relapse Rates After Autologous Transplantation

During follow-up periods (median, 870 days; range, 126-2913 days), 51 patients (61%) relapsed or had a progressive disease (Table 2). Both relapse rates as well as time to relapse after autologous transplantation were very similar among patients with and without ES (Table 3). The relapse rate was 61% in both groups, and the median time to relapse was 365 versus 367 days, respectively. The relapse rates were similar regardless of disease stage. However, there was a difference between the 2 groups in the sites and patterns of relapse. Relapse occurred in visceral organs in 17 (85%) of 20 patients who had ES versus 15 (48%) of 31 patients without ES ($P = .01$). There was no difference in survival rates for patients with ES who did (n = 23) or did not (n = 11) receive steroid therapy (52% versus 38%, $P = .84$). The only patients receiving high-dose and long-term steroids were those with pulmonary diffuse alveolar hemorrhage, and they were long-term survivors.

Table 3. *Relapse Rates in Breast Cancer Patients With and Without Engraftment Syndrome (ES)*

Disease Stage	No. With Relapse/No. of Patients (%) With ES	Without ES	P*
II	1/3 (33)	1/8 (12)	.42
III	2/9 (22)	9/28 (32)	.57
IV	17/21 (81)	21/24 (87)	.54
Total	20/33 (61)	31/51 (61)	NS

*Statistical analysis was performed using the Fisher exact test to compare the relapse rates of 2 groups of patients (with ES versus without ES).

Effect of ES on Disease-Related Mortality

The mortality and survival rates were found to be different in the 2 groups (patients with ES versus patients without ES). At the time of this analysis, 17 (52%) of 33 ES patients had died compared with only 16 (31%) of 51 patients without ES (Table 4, Figure 1). Although this difference in mortality rate is clear, it has not reached statistical significance ($P = .08$).

Analysis of the data of the patients who had relapsed after autologous transplantation showed that the patients with a history of ES had significantly worse prognoses. As mentioned above (Table 2), a total of 51 patients (61%) had relapsed. Of those patients, 20 had histories of ES and 17 (85%) of 20 have died due to disease progression, whereas 16 (51%) of the other 31 patients who had no history of ES died due to disease progression ($P = .008$) (Figure 2). Univariate analysis showed that in addition to ES, stage of disease at transplantation, remission status at transplantation (complete versus partial remission), number of prior treatments, and visceral relapse were associated to a variable degree with increased mortality rates, whereas estrogen/progesterone receptor positivity was associated with better prognoses (Table 5). In multivariate analysis, ES was independently associated with increased mortality ($P = .04$; hazard ratio, 2.1; 95% confidence interval, 1.002-4.62) when analyzed in association with number of prior treatments and progesterone receptor status (Table 6).

DISCUSSION

ES or autoaggression syndrome described after autologous BMT/PBSCT was proposed to be a GVHD-like syndrome. ES manifests mainly with skin rash (with biopsy findings consis-

Table 4. *Difference in Mortality Rates in Breast Cancer Patients With Engraftment Syndrome (ES) Compared With Patients Without ES*

Disease Stage	Mortality, No. of Patients Who Died/No. of Patients (%) Overall	With ES	Without ES	P*
Stage II	1/11 (9)	1/3 (33)	0/8 (0)	.08
Stage III	8/28 (29)	2/9 (22)	6/19 (32)	.60
Stage IV	24/45 (53)	19/21 (67)	10/24 (42)	.09
Total	33/84 (39)	17/33 (52)	16/51 (31)	.08

*Statistical analysis was performed using the Fisher exact test to compare the mortality rates of 2 groups of patients (with ES versus without ES).

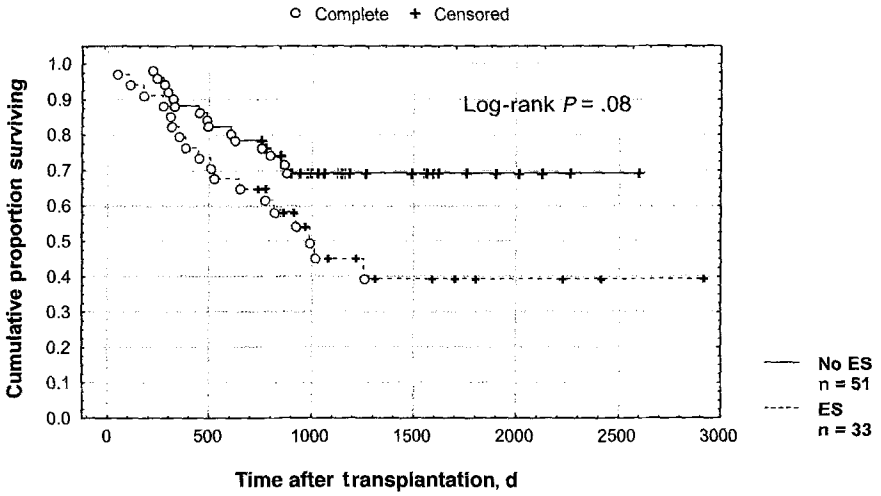

Figure 1. Kaplan-Meier curves showing the difference in survival rates of patients who developed engraftment syndrome (ES) after transplantation (n = 33; broken line) versus patients who did not develop ES after transplantation (n = 51; solid line).

tent with acute GVHD), noninfectious fever, and, in some patients, diarrhea, platelet transfusion refractoriness, and pulmonary alveolar hemorrhage [18-21]. ES was reported to respond to steroid treatment. Although the pathogenesis of ES is not known, it has been hypothesized that mechanisms that normally safeguard the self-tolerance properties of the immune system are suppressed by high-dose chemotherapy. Hence, during early stages of engraftment, autoreactive effector T cells may be present in excess quantities under uninhibited conditions, leading to the various manifestations of this syndrome [18,22]. It has been observed that patients with breast cancer are at higher risk of developing ES compared to lymphoma patients and that such a difference could be attributed to an immune system that is likely to be less suppressed in breast cancer patients [18]. Because of the possible analogy of ES to acute GVHD, we investigated any evidence for GVT effect among breast cancer patients who developed ES versus those who did not. In this analysis, we found a 39% ES incidence rate among 84 patients undergoing autografting. There was no difference in relapse rates (approximately 61%) for patients with or without ES, with median follow-up of 871 days. This finding raises the issue of autologous GVHD and its effectiveness in mediating antitumor response and reducing the high relapse rates seen after autografting [11]. Because breast cancer cells express major histocompatibility-complex class II antigens [23,24], studies aiming at inducing autologous GVHD after autografting in breast cancer patients have been described. Cyclosporin, interferon α and γ, and interleukin 2 have been used in various combinations to induce autologous GVHD [15-17,25,26]. The results of these studies and others done in other diseases have failed to show any significant improvement in relapse or survival rates, despite the induction of histologically proven skin GVHD in high proportions of the patients studied. Thus, the results of our analysis are in agreement with the results of those studies, suggesting no significant GVT effect of this GVHD-like ES. Other than the clinical symptoms, the only evidence suggesting that ES is like GVHD is found in pathological results of skin biopsies that are consistent with GVHD [18].

The most significant, and unexpected, results of our present study are the findings that relapsed patients who

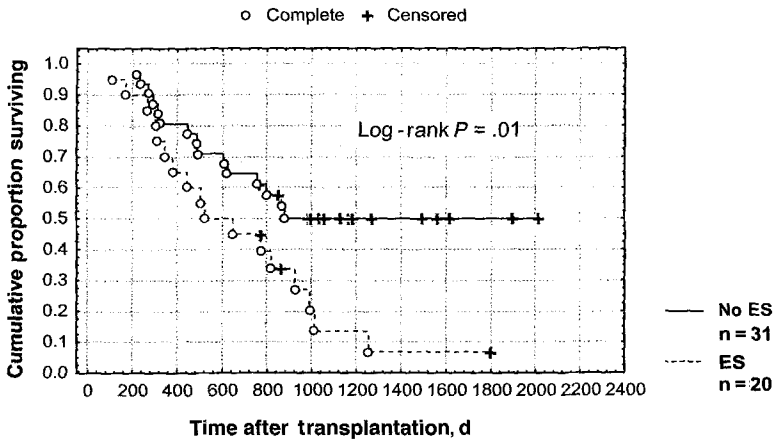

Figure 2. Kaplan-Meier curves showing the difference in survival rates of patients who had relapsed after transplantation based on whether they had developed engraftment syndrome (ES) (n = 20; broken line) or not (n = 31; solid line).

Table 5. *Effects of Various Prognostic Factors on Mortality in Breast Cancer Patients (Univariate Analysis)*

Prognostic Factor	P	Hazard Ratio	95% Confidence Interval
Engraftment syndrome	.089	1.809	0.914-3.582
Disease stage	.0058	2.945	1.367-6.346
Remission status	.0015	3.238	1.568-6.688
No. of prior treatments	.0053	2.762	1.353-5.637
Estrogen receptor positive	.0706	0.505	0.241-1.059
Progesterone receptor positive	.0311	0.417	0.189-0.924
Visceral relapse	.0835	1.980	0.914-4.291

had ES after autografting had more widespread (80% visceral) aggressive disease resulting in significantly higher mortality rates (85%) and shorter median survival times (586 days) compared with those who did not develop ES (48% and 847 days, respectively; $P = .01$). The reason for such differences is most likely not related to disease stage or behavior, because the trend for poorer outcomes in patients with ES has been observed in patients with advanced as well as less advanced disease during the time of transplantation. Conversely, ES might indicate widely micrometastatic disease at the time of transplantation, and, therefore, ES may be a manifestation of the immune response against an existing residual tumor. Although such an explanation is highly speculative, multivariate analysis has shown that the occurrence of ES is an independent prognostic factor for bad outcome after autografting in our breast cancer patient population. Until the exact pathophysiology of ES is elucidated, it will be difficult to explain such grim outcomes for those breast cancer patients. Another theoretical reason for the difference in survival rates after relapse could be differences in the treatment of relapse used in each group (with ES versus without ES). Although this issue was not studied here, most patients were treated with standard chemotherapy and/or radiotherapy by their referring physicians, so it is more likely that the extent of disease rather than the treatment contributed to the difference in survival.

In summary, we report a high incidence of ES with no effect on relapse rate in breast cancer patients undergoing autologous transplantation. In addition, those patients who develop ES have higher mortality rates and significantly shorter survival times than patients who do not develop ES, and thus ES may be a marker for high-risk groups of breast cancer patients that should be the target of further evaluation and treatment after autografting. Although these observations are intriguing, further validation in larger studies or studies from other centers will be important before firm conclusions can be reached.

Table 6. *Engraftment Syndrome as a Predictive Factor for Mortality in Breast Cancer Patients Undergoing Autologous Transplantation (Multivariate Analysis)*

Prognostic Factor	P	Hazard Ratio	95% Confidence Interval
Engraftment syndrome	.0494	2.152	1.002-4.621
No. of prior treatments	.0005	4.418	1.909-10.225
Progesterone receptor status	.0051	0.300	0.129-0.697

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